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PHYTOCHEMICAL ANALYSIS OF SOME SELECTED SPICES

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ABSTRACT:
Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Herbs and spices have been used during the middle Ages for flavouring, food preservation, and/or medicinal purposes. The present study was carried out on the four spices, *Cinnamomum verum, (*Cinnamon), *Illicium verum* (Star anise), garlic cloves (Allium sativum), and dried turmeric powder (Curcuma longa), *Capsicum Annuum L. Coriandrum sativum L. dhane Piper nigrum L.-Mire Ferula asafoetida L.-Hing, Trigonella foenum-graecum L - Methi, Zingiber officinale Rosc.-Sunth* to determine their phytochemical constituents and were proved to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.

Keywords: Spices, Phytochemicals, *Cinnamomum verum* (Cinnamon), *Illicium verum.*

INTRODUCTION:

Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C. and 800 B. C. & given many references of medicinal plants. One of the remotest works in traditional herbal medicine is "*Virikshayurveda*", compiled even before the beginning of Christian era. "*Rig Veda*", one of the oldest available literatures written around 2000 B. C. mentions the use of Cinnamon (*Cinnamomum verum*), Ginger (*Zingiber officinale*), Sandalwood (*Santalum album*) etc. not only in religious ceremonies but also in medical preparation

Aims and objective:

[1]. Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body

[2] .Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self-experiment. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant (Holiman, 1989). According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs.

[3].The medicinal value of Spices, which include leaves (coriander,mint), buds (clove), bulbs (garlic, onion), fruits (red chili, black pepper), stem (cinnamon), rhizomes (ginger), star anise, cinnamon(bark) and other plant parts, have been defined as plant substances from indigenous or exotic origin,aromatic or with strong taste, used to enhance the taste of foods. Herbs and spices have been used during the middle Ages for flavoring, food preservation, and/or medicinal purposes. Only a small percentage of plants species have been investigated phytochemically and the fraction submitted to biological screening is even smaller [4]. Several studies have attributed the antimicrobial, antioxidant and pharmaceutical properties of spices and herbs to their phenolic compounds

Table. 1A: Review of Concerned Literature.

Phytoch-emicals	Activity	Mechanism of action
Quinones	Antimicrobial	Binds to adhesins, complex with cell wall, inactivates enzymes
Flavonoids	Antimicrobial Antidiarrhoeal	Complex with cell wall, binds to adhesins, Inhibits release of autocoids and prostaglandins, Inhibits contractions caused by spasmogens, Stimulates normalization of the deranged water transport across the mucosal cells, Inhibits GI release of acetylcholine
Polyphenols and Tannins	Antimicrobial Antidiarrhoeal Anthelmintic	Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation, Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin

		to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action. Increases supply of digestible proteins by animals by forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in G.I. metabolism
Coumarins	Antiviral	Interaction with eucaryotic DNA
Terpenoids and essential oils	Antimicrobial Antidiarrhoeal	Membrane disruption Inhibits release of autocoids and prostaglandins
Alkaloids	Antimicrobial Antidiarrhoeal Anthelmintic	Intercalates into cell wall and DNA of parasites. Inhibits release of autocoids and prostaglandins. .Possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing paralysis
Lectins and Polypeptides	Antiviral	Blocks viral fusion or adsorption, forms disulfide bridges
Glycosides	Antidiarrhoeal	Inhibits release of autocoids and prostaglandins
Saponins	Antidiarrhoeal Anticancer Anthelmintic	Inhibits histamine release in vitro . Possesses membrane permeabilizing properties. Leads to vacuolization and disintegration of teguments
Steroids	Antidiarrhoeal	Enhance intestinal absorption of Na+ and water

MATERIAL AND METHODS:

Phytochemical Analysis

Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignins.

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion,decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfl eurage (cold fat extraction) may be employed. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermo-microdistillation and molecular distillation

The basic parameters influencing the quality of an extract are

1. Plant part used as starting material
2. Solvent used for extraction
3. Extraction procedure

Effect of extracted plant phytochemicals depends on

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depends upon

1. Type of extraction
2. Time of extraction
3. Temperature
4. Nature of solvent
5. Solvent concentration
6. Polarity

Plant material:

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from anypart of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either

randomly or by following leads supplied by local healers in geographical areas where the plants are found. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Other researchers dry the plants in the oven at about 40°C for 72 h. In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for bioactive compounds possessing antimicrobial properties

Choice of solvents:

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted

The various solvents that are used in the extraction procedures are:

Water: Water is universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics only important as antioxidant compound

Chloroform: Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents].

Ether: Ether is commonly used selectively for the extraction of coumarins and fatty acids

Solvents used for active component extraction-

Water	Chloroform	Ether	Acetone
Tannins	Terpenoids	Alkaloids	Flavonols
Saponins		Fatty acids	
Terpenoids		Coumarins	
Polypeptides			
Lectins			
Anthocyanins			

Methods of extraction:

Variation in extraction methods usually depends upon:

1. Length of the extraction period,
2. Solvent used,
3. pH of the solvent,
4. Temperature,
5. Particle size of the plant tissues

The solvent-to-sample ratio The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal

Extraction procedures:

Soxhlet extraction: Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds .

Phyto-chemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods. to detect the bioactive compounds like alkaloids, tannins, phenols, steroids, flavonoids, saponins (Trease et al, 1989).

Method of extraction:
Solvent – Petroleum ether, Methanol
Method – Maceration

Procedure:
Plant part (leaf) powder was weighed 500 gm and kept in a container in contact with pet ether for seven days, with vigorous shaking at regular interval. Material was filtered a first with muslin cloth and then with filter paper. Filtrate was collected and dried in water bath till no further reduction in mass of extract was observed. Dried extract was weighed and packed in air tight containerAnd the marc was air dried then kept in a container in contact with methanol for seven days, with vigorous shaking at regular interval. Material was filtered a first with muslin cloth and then with filter paper. Filtrate was collected and dried in water bath till no further reduction in mass of extract was observed. Dried extract was weighed and packed in air tight container

Spices:
Four samples of spices *Cinnamomum verum* (Cinnamon), *Illicium verum* (Star anise, garlic cloves (*Allium sativum*), and dried turmeric powder (*Curcuma longa*), were used in this study

Preparation of ethanolic extracts:
Samples of spices were pulverized and extracted twice in ethanol (1:10 w/v) at room temperature for 48 hrs and filtered. The filtrates were concentrated to dryness under reduced conditions at room temperature. Dried extracts were then suspended in dimethyl sulfoxide (DMSO) for further use.

Phytochemical Screening Test:

A. Test for carbohydrates:
Equal volumes of Benedict's reagent and test solution were mixed in attest tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

B. Tests for Proteins: Xanthoproteic test:
To 1ml of extract, 1ml of conc.H2SO4 was added. This resulted in the formation of white precipitate which on boiling turned yellow. On addition of NH4OH, yellow ppt. turned orange.

C. Test for Steroids: Salkowski Test:
To 2ml of aqueous extract, 2ml of chloroform and 2ml of conc.H2SO4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

D. Tests for alkaloids: The aqueous extract was evaporated in a test tube. To the residue dilute HCl was added shaken well and filtered. With the filtrate following tests were performed.

Hager's Test- To the 2-3ml of filtrate hager's reagent was added. Yellow ppt was formed showing the presence of alkaloids.

Mayer's Test- To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

With tannic acid- To 1ml of extract add 2-3 drops of the tannic acid solution reagent, appearance of amorphous or crystalline precipitate represents the presence of alkaloid.

F. Test for saponins- Drug extract was shaken vigorously with water. No persistent foam was formed.

G. Test for Tannins- For 2ml of extract add few drops of 1% lead acetate. A yellowish precipitate showed the presence of tannins.

H. Test for Anthocyanins- 2ml of aqueous extract is added to 2ml of 2N HCl and ammonia. The appearance of pink red turns blue violet indicates the presence of anthocyanins.

I. Test for coumarins- 3ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow color indicates the presence of coumarins.

OBSERVATION AND RESULTS:

Table. 1: Preliminary qualitative phytochemical analysis of some spices (salvent- water)

S.N.	Name of plants	Phytochemical test									
		Al	St	Ph	Tan	Sap	An	Cou	Car	Pro	AA
1	*Capsicum Annuum L.*	-	-	-	-	-	-	+	-	-	-
2	*Illicium Verum Hook Star-annis*	-	-	-	-	+	-	-	-	-	-
3	*Cinnamomum versum Dalchini*	-	-	-	-	+	-	-	+	-	-
4	Allium sativum Garlic	-	-	-	-	+	-	-	-	+	+
5	*Curcuma longa Haldi*	-	-	-	-	-	-	-	+	-	-
6	*Coriandrum Sativum L. Dhane*	-	-	-	+	-	-	+	-	-	-
7	*Piper Nigrum L -Mire*	-	-	-	-	+	-	-	-	-	-
8	*Ferula Asafoetida L- Hing*	-	-	-	-	+	-	-	-	+	+
9	*TrigonellaFoenum-Graecum L-Methi*	-	-	+	+	-	+	+	-	+	-
10	*Zingiber Officinale Rosc. Sunth*	-	-	-	-	+	-	-	-	-	-

Table. 2: Preliminary qualitative phytochemical analysis of some spices (salvent-Chloroform)

S. N.	Name of plants	Phytochemical test									
		Al	St	Ph	Tan	Sap	An	Cou	Car	Pro	AA
1	*Capsicum Annuum L.*	-	-	-	-	-	-	-	-	-	-
2	*Illicium Verum Hook Star-annis*	-	-	-	-	-	-	-	-	-	-
3	*Cinnamomum versum Dalchini*	-	-	-	-	-	-	+	+	+	-
4	Allium sativum Garlic	-	-	+	-	-	-	-	-	-	-
5	*Curcuma longa Haldi*	-	+	-	-	-	-	-	+	-	-
6	*Coriandrum Sativum L. Dhane*	-	-	-	-	-	-	+	-	-	-
7	*Piper Nigrum L –Mire*	-	-	-	-	-	-	-	-	-	-
8	*Ferula Asafoetida L- Hing*	-	-	-	-	-	-	-	-	+	+
9	*TrigonellaFoenum-GraecumL-Methi*	-	-	-	-	-	-	-	-	-	-
10	*Zingiber Officinale Rosc. Sunth*	-	+	-	-	-	-	-	-	-	-

Abbreviations
Al: Alkaloids **St**. Steriods; **Ph**: Phenols; **Tan**: Tannins; **Sap**: Saponins; **An**: Anthocyanin,.**Cou**; coumarin. **Car**; Carbohydrates ;**Pro;** Proteins AA: Aminoacids, **(+)** Indicate the presence of phytochemicals and **(-)** Indicate the absence of phytochemicals

Table. 3: Preliminary qualitative phytochemical analysis of some Spices (Salvent-Ethanol)

S.N.	Name of plants	Phytochemical test									
		Al	St	Ph	Tan	Sap	An	Cou	Car	Pro	A A
1	*Capsicum Annuum L.*	-	+	-	-	-	-	-	-	+	+
2	*Illicium Verum Hook Star-annis*	-	-	-	+	-	-	-	-	-	-
3	*Cinnamomum versum Dalchini*	-	+	+	-	+	-	-	+	-	-
4	Allium sativum Garlic	-	-	+	-	-	-	+	-	+	+
5	*Curcuma longa Haldi*	-	+	-	+	-	-	_	+	-	-
6	*Coriandrum Sativum L. Dhane*	-	-	-	+	-	-	+	-	-	-
7	*Piper Nigrum L –Mire*	+	-	-	-	+	-	+	+	+	-
8	*Ferula Asafoetida L- Hing*	-	-	-	-	+	-	-	-	+	+
9	*TrigonellaFoenum-GraecumLmethi*	+	+	-	+	+	+	+	-	-	+
10	*Zingiber Officinale Rosc. Sunth*	+	-	-	+	+	-	+	-	-	-

Table. 4: Preliminary qualitative phytochemical analysis of some Spices- Result

S.N.	Name of plants	Phytochemical test									
		Al	St	Ph	Tan	Sap	An	Cou	Car	Pro	A A
1	*Capsicum Annuum L.*	-	+	-	-	-	-	+	-	+	+
2	*Illicium Verum Hook Star-annis*	-	-	-	+	+	-	-	-	-	-
3	*Cinnamomum versum Dalchini*	-	+	+	-	+	-	+	+	+	-
4	Allium sativum Garlic	-	-	+	-	+	-	+	-	+	+
5	*Curcuma longa Haldi*	-	+	-	+	-	-	_	+	-	-
6	*Coriandrum Sativum L. Dhane*	-	-	-	+	-	-	+	-	-	-
7	*Piper Nigrum L –Mire*	+	-	-	-	-	-	+	+	+	-
8	*Ferula Asafoetida L- Hing*	-	-	-	-	-	-	-	-	-	+
9	*TrigonellaFoenum-GraecumLmethi*	+	+	+	+	+	+	+	-	+	+
10	*Zingiber Officinale Rosc. Sunth*	+	+	-	+	+	-	+	-	-	-

Abbreviations
Al: Alkaloids **St**. Steriods; **Ph**: Phenols; **Tan**: Tannins; **Sap**: Saponins; **An**: Anthocyanin,.**Cou**; coumarin. **Car**; Carbohydrates ;**Pro;** Proteins AA: Aminoacids, **(+)** Indicate the presence of phytochemicals and **(-)** Indicate the absence of phytochemicals

RESULT AND DISCUSSION:

The present study carried out on the four spices i.e., *Cinnamomum verum* (Cinnamon), *Illicium verum* (Star anise), *Allium sativum* (garlic cloves), and Curcuma longa(turmeric powder), were used in this study and revealed the presence of medicinal active constituents. The phytochemical active compounds of these spices were qualitatively analyzed separately and the results are presented in Table 1.In these screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides, phenols shows different types of results in different solvents. The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hyper cholesterolemia and antibiotic properties Steroids and triterpenoids show the analgesic for central nervous system activities. Phytochemical screening of the various extracts of *Cinnamomum verum* (Cinnamon), *Illicium verum* (Star anise), *Allium sativum* (garlic cloves), and Curcuma longa(turmeric powder)were used to study the presence of contained alkaloids, flavonoids, steroids, saponins, tannins and triterpenoid and also have various medicinal values such as anti-inflammatory, anti diabetic and analgesic activities and for central nervous system activity. The importance of alkaloids,

saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by (Kubmarawa Mensah, 2008)

CONCLUSION:

The spices have been screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.

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